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Award Number: W81XWH-04-1-0083

TITLE: Positional Cloning of an Ashkenazi Jewish Hereditary Prostate Cancer Susceptibility Locus

PRINCIPAL INVESTIGATOR: Danielle Friedrichsen, Ph.D.

CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Research Center
Seattle, WA 98109-1024

REPORT DATE: January 2006

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-01-2006	Final	1 FEB 2004 - 31 DEC 2005

4. TITLE AND SUBTITLE
Positional Cloning of an Ashkenazi Jewish Hereditary Prostate Cancer Susceptibility Locus

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0083

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Danielle Friedrichsen, Ph.D.

E-mail: dfriedri@fhcrc.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Fred Hutchinson Cancer Research Center
Seattle, WA 98109-1024

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The fellowship aimed to confirm the 7q11-21 linkage result, to identify the founder haplotype and to clone the disease-associated mutation. The final research accomplished has: 1) confirmed the 7q11-21 linkage result by analysis of additional microsatellite markers in the 37.6 cM region and a combined genome-wide scan of 36 Jewish HPC families (empirical P=0.006); 2) defined the minimal recombination region for the 18 PROGRESS Jewish families as a 5.7 cM interval with 21 RefSeq genes; 3) sequenced almost all of the exons in the MRR (119/134, 88%); 4) genotyped key individuals from the 18 Jewish families on the Affymetrix 100K SNP chips (163 total chips); 5) built the chromosome 7 haplotypes in each family with the 7,069 SNPs from the SNP chips; 6) designed and wrote analysis scripts to identify haplotype patterns enriched in the affecteds; 7) investigated 22 regions further by genotyping 113 potentially informative SNPs from the HapMap project; 8) generated a large volume of chromosome 7 genetic information with a total of 73 microsatellites, 7,069 chip SNPs, and 727 sequenced SNPs or amplicons with one or more exons.

15. SUBJECT TERMS
Hereditary Prostate Cancer, Susceptibility Genes, Linkage Analysis, Positional Cloning, Ashkenazi Jewish Families

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
					USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
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INTRODUCTION

Hypothesis: Ashkenazi Jews represent a well-defined homogenous population with founder mutations already identified in other cancer susceptibility genes. Defining homogenous subsets of hereditary prostate cancer (HPC) families is key for successful linkage analysis due to the substantial genetic heterogeneity of prostate cancer (PC). Therefore, I propose to investigate HPC by linkage analysis in Ashkenazi Jews in the hopes of identifying and positionally cloning a HPC susceptibility gene. Support for this hypothesis is evident by my preliminary analysis of 17 Jewish families, which identified a region of suggestive linkage on chromosome 7 near the centromere.

Specific Aims: (1) Confirm linkage results by analysis of additional markers spanning the minimal critical region. (2) Define the minimal recombination region by mapping recombination events within Ashkenazi Jewish families. (3) Identify the Jewish HPC founder haplotype. (4) Prioritize candidate genes for mutation detection in the Jewish HPC region by their location, expression pattern, and biological relevance. (5) Screen for disease-associated mutations and test findings in our other HPC families with linkage to the Jewish HPC locus.

Relevance: Cloning HPC susceptibility genes are directly relevant to the main objectives of the PC research program. Even though only a small percent of PC cases overall will be due to mutations in any one HPC susceptibility gene, 43% of men diagnosed under the age of 55 have an inherited susceptibility gene. Identification of these genes increases our understanding of PC etiology in several ways. First, identification of susceptibility genes leads to insights into cellular mechanisms important in the disease progression. For example, the cloning of *BRCA1* and subsequent discovery that the BRCA1 protein interacts with Rad51 revealed that cellular DNA repair mechanisms are involved in breast cancer susceptibility. Second, susceptibility genes may highlight previously unknown biological pathways involved in PC. Third, the obvious relevance is for Ashkenazi Jews with a family history of PC. The identification of a HPC founder mutation in this population potentially allows for genetic testing and could lead to early detection of the disease. Finally, mutations in the Jewish HPC susceptibility gene may also be important in men in the general population, as is the case for the breast cancer susceptibility genes, *BRCA1* and *BRCA2*.

BODY

The following summarizes the objectives of this postdoctoral fellowship and the work accomplished by the end of the funding period (12/31/05).

Aim 1. Confirm linkage results by analysis of additional markers spanning the minimal critical region (Months 1-3):

Task 1a: Select and optimize microsatellite markers spaced every 3 cM (Month 1). The minimum critical region included five markers, covering 37.6 cM, and was from markers D7S2846 to D7S2212. Twelve markers spaced every 3 cM (where possible)

were selected from the genetically mapped microsatellites on the UCSC genome browser (www.genome.ucsc.edu) and optimized in this 37.6 cM region, which is three more markers than originally proposed.

Task 1b: Genotype these markers in the 94 individuals from 17 collected and well characterized Ashkenazi Jewish families, including the 50 affected men (Month 1-3). All twelve microsatellite markers were successfully genotyped in all 93 individuals with blood samples from 18 Ashkenazi Jewish families. This dataset includes one additional well-characterized Jewish family compared to the original result for a total of 51 genotyped affected men.

Task 1c: Perform statistical analysis to confirm preliminary results (Month 3). The Pedcheck program was used to identify genotyping errors inconsistent with Medelian inheritance, where any errors discovered were corrected in the dataset. Then, both parametric and nonparametric linkage analyses were performed using the GENEHUNTER and Merlin programs.

Table 1: NPL *P* values for the 18 Jewish families with the 12 additional mini-scan markers

	Chromosome 7	
Marker	cM Position	NPL *P* value
D7S2846	57.87	0.18130
D7S2192	61.58	0.07048
TAT028	63.74	0.05817
D7S667	66.78	0.02199
D7S1818	69.46	0.00145
D7S674	70.02	0.00122
D7S2422	71.92	0.00116
D7S1830	72.85	0.00229
D7S2552	74.82	0.00596
D7S499	76.14	0.01078
D7S473	76.88	0.01310
D7S3046	80.40	0.00557
D7S2435	80.63	0.00629
D7S1816	82.43	0.00448
D7S2518	86.52	0.00359
D7S2204	89.12	0.00302
D7S2443	91.25	0.00557
D7S2212	95.19	0.00251
D7S820	96.63	0.00216
D7S821	105.06	0.00710
D7S1799	111.87	0.03451
D7S2847	122.23	0.12010

In the original analysis of the 17 Jewish families with the genome-wide scan microsatellite markers, there was a region of suggestive linkage on chromosome 7 near the centromere. For seven consecutive markers, D7S1818 to D7S821, the NPL score was greater than 2.69 (nominal *P* value < 0.06). A maximum NPL score of 3.46 ($P < 0.001$) was observed at 72.8 cM. The result remains consistent after the addition of 12 mini-scan microsatellite markers within the minimal critical region (D7S2846 to D7S2212; Table 1). The NPL score is greater than 2.3 (nominal P value < 0.02) for 16 consecutive markers. The maximum NPL score was 3.36 at 71.9 cM, and the maximum multipoint LOD was 2.47 at 96.6 cM.

Finally, the chromosome 7 linkage result was confirmed by our combined genome-wide scan analysis 36 Jewish families, including the original 17 Jewish families from the PROGRESS study and 19 Jewish families from Johns Hopkins University (Friedrichsen et al., 2003). All available family members, including 94 affected men, were genotyped at markers distributed across the genome with an average interval of less

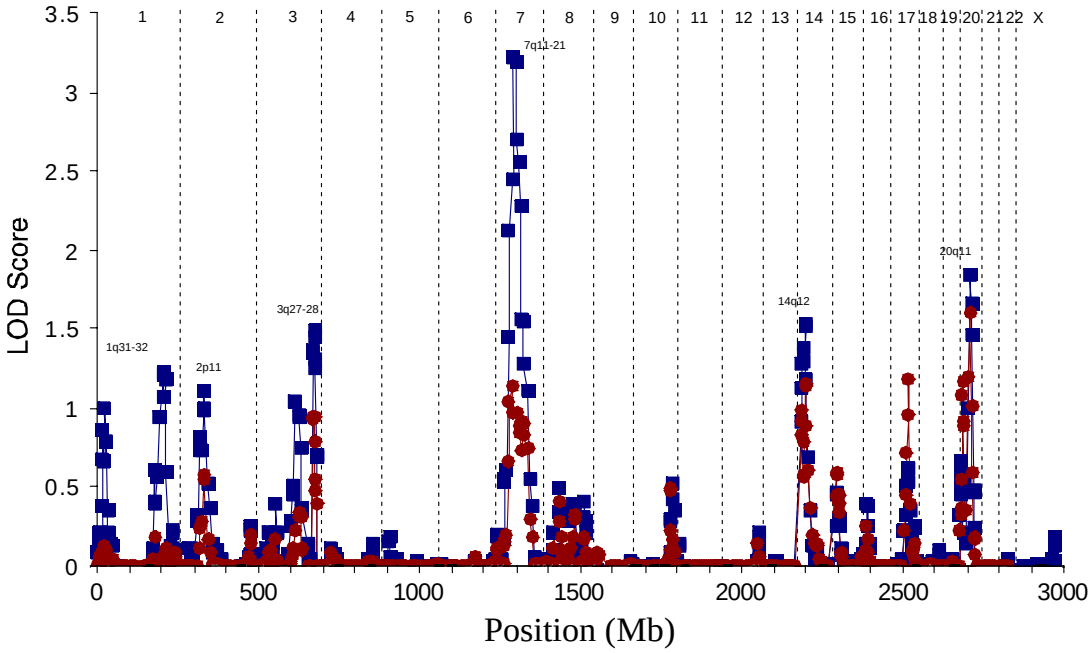

Figure 1: Results of Genome-Wide Scan in the 36 Jewish Families Suggest a Hereditary Prostate Cancer loci at 7q11-21.

Microsatellite markers were genotyped across the whole genome (PROGRESS 441 markers, JHU 406 markers). Allele-sharing LOD scores (■) were implimented by Merlin and HLOD scores from multipoint parametric analysis using a 2-liability class model (◆) were analyzed by Genehunter. The chromosome number is designated at the top of the graph.

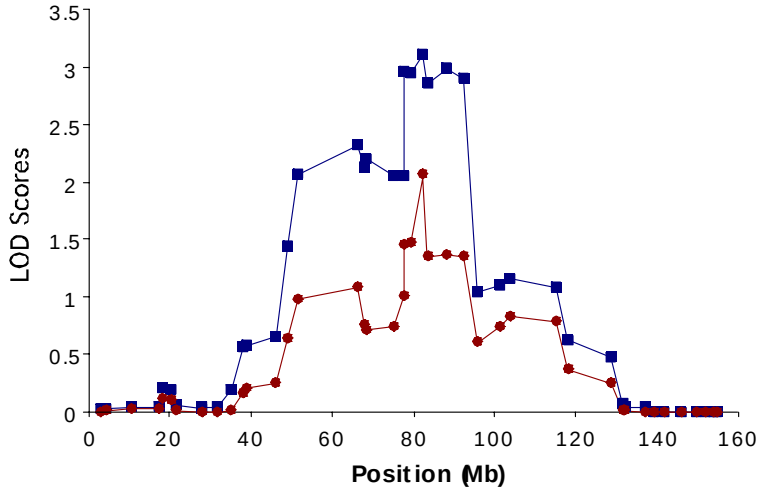

Figure 2: Fine Mapping Multipoint Linkage Results Support the 7q11-21 Hereditary Prostate Cancer locus.

The final dataset included 11 markers across the 7q11-21 peak where genotypes were available for both PROGRESS and JHU families, including three new markers. Allele-sharing LOD scores (■) were implimented by Merlin and HLOD scores from multipoint parametric analysis using a 2-liability class model (◆) were analyzed by Genehunter.

than 10 cM. Nonparametric multipoint linkage analyses were the primary approach, although parametric analyses were performed as well. Our strongest signal was a significant linkage peak at 7q11-21 (Figure 1), with a nonparametric linkage (NPL) score of 3.01 ($P = 0.0013$). Simulations indicated that this corresponds to a genome-wide empirical $P = 0.006$. All other regions had NPL P values ≥ 0.02. After genotyping additional mini-scan markers within the 7q11-21 peak (Figure 2), the NPL score increased to 3.35 ($P = 0.0004$) at D7S634 with an allele-sharing LOD of 3.12 ($P = 0.00007$).

Aim 2. Define the minimal recombination region by mapping recombination events within Ashkenazi Jewish Families (Months 4-6):

Task 2a: Select and optimize microsatellite markers spaced every 1 cM at both the proximal and distal ends of the region (Month 4). The original minimal recombination region was 37.6 cM from markers D7S2846 to D7S2212. Recombination events in four families delimited the proximal end of the minimal critical region between markers D7S2846 and D7S1818 (57.8-69.6 cM). After addition of the 12 markers in Aim 1, evidence for the proximal end was now a recombination in one family between D7S2518 and D7S2204. Four additional microsatellite markers spaced every 1 cM (or less) were selected and optimized. The distal end of the region was set by recombinations found in two families between D7S2204 and D7S2212 (91.0-95.4 cM). The Aim 1 markers did not shift the location of these two families recombination events. Eight additional markers were selected and optimized to add information to the distal end of the region for a total of twelve Aim 2 markers.

Task 2b: Genotype these markers in the 6 Ashkenazi Jewish families (39 individuals) with informative recombination events (Month 4-6). These twelve microsatellite markers were genotyped in all 93 individuals from the 18 Jewish families. This was done because genotyping the markers in all the families could uncover thus far undetected recombination events as the Aim 1 markers did in one of the families. Also, genotyping these markers in all the families will be helpful in Aim 3.

Task 2c: Locate the recombination events in the 6 informative families to set the minimal recombination region (Month 6). The minimal recombination region for the 18 PROGRESS Jewish families is 5.7 cM. This is defined by one recombination on the proximal and distal edge and covers 4.5 Mb with 21 RefSeq genes within the region. With two recombinations defining either edge, the region expands to 29.2 cM covering 28.5 Mb and would contain 80 additional RefSeq genes for a total of 101 RefSeq genes.

The minimal recombination region is similar when 18 of the original 19 JHU Jewish families from the combined genome-wide scan analysis were included with the 18 PROGRESS families. Twenty-eight additional microsatellite markers were genotyped in the 18 JHU families to define recombinations within these families. With the addition of the JHU families, there is a conflict because where the affecteds within a family share a chromosome does not overlap at a single marker in all the linked families. Thus, there is evidence for two minimal recombination regions. Using three recombination events on either edge, the first minimal recombination region includes 26 families and is the same as the 5.7 cM region defined in the PROGRESS families alone with 21 RefSeq genes in

the region. The second minimal recombination region is toward the p-terminus and it includes 25 families covering 37 Mb with 131 RefSeq genes in the region.

Aim 3. Identify the Ashkenazi Jewish HPC haplotype (Months 5-11):

Task 3a: Select the informative marker or markers from Task 1b, where the affected haplotype in all or the majority of families is the same allele (Month 5). After Aim 2, the minimal recombination region (MRR) in the 18 PROGRESS families was only 5.7 cM with 21 RefSeq genes. Since the region was so small, the approach in identifying the founder haplotype and/or founder mutation was modified by genotyping available microsatellite markers as well as sequencing coding regions of genes (Aim 5) at the same time. The three remaining genetically mapped microsatellites located in this 5.7 cM region were selected for genotyping.

Task 3b: Genotype the remaining microsatellite markers in this region in the 17 Ashkenazi Jewish families (Month 5-7). The three additional markers were genotype in all 93 individuals from the 18 PROGRESS Jewish families.

Task 3c: Identify and genotype SNPs such that an informative genetic marker is located every 0.1-0.3 Mb (Month 6-9). For the MRR, SNPs were identified using dbSNP. (This aspect of the project was completed before the release of the HapMap data.) SNPs located near intron-exon boundaries were preferentially selected and the SNP as well as the exon was sequenced. This allowed for the screening for SNPs and potential mutations at the same time. In the MRR, 119 out of the 134 exons (88%) were sequenced directly. No obvious mutation was identified. Including an additional 30 SNPs, which were not next to an exon, there was an informative microsatellite or SNP every 0.3 Mb in the MRR, with only one gap between 0.2-0.3 Mb. There was no obvious founder haplotype discovered in this 5.7 cM region.

Task 3d: Determine the location of the Ashkenazi Jewish HPC haplotype and genotype additional SNPs if necessary to define the proximal and distal ends of the haplotype (Month 8-11). The Jewish HPC haplotype was not discovered in the analysis of the 5.7 cM minimal recombination region (MRR) with a microsatellite or SNP marker every 0.3 Mb or less. This could be for several reasons. The MRR is defined by one recombination event on either edge. As mentioned in the fellowship, individuals with sporadic prostate cancer in these HPC families would misdirect refinement of the minimal recombination region. Therefore, defining the MRR by three recombination events on either edge may be a necessary method in diseases where the rate of sporadic disease is more common. Analysis of the 36 PROGRESS and JHU families indicated two different MRRs defined by three recombination events on either edge (Aim 2). Therefore, I extended the region and repeated Task 3a and 3b again for the larger MRR. Alternatively, an informative marker every 0.3 Mb or less may not be dense enough to identify the founder haplotype. This density was selected because studies of several cancer susceptibility genes, including *BRCA1*, *BRCA2* and *MSH2*, have identified founder haplotypes in the Ashkenazi Jewish population, and these haplotypes have been 0.5-1.0 Mb or greater. It is possible that the HPC mutation is an older mutation, which would result in a smaller founder haplotype surrounding the mutation. Genotyping the 18 Jewish families on the Affymetrix 100K SNP chips (described below) directly address this issue because the 100K SNP chips have a SNP every 50 kb on average.

The founder haplotype search region was expanded to a 50.3 cM region with three recombination events on either boundary in the 18 PROGRESS Jewish families. (This step of the project was completed before the analysis of the 18 JHU families in Aim 2.) Additional microsatellites, SNPs and exons were sequenced within this 50.3 cM to discover informative markers in the 18 Jewish families. During this phase, 46 microsatellite markers, 17 SNPs from dbSNP and 366 amplicons with one of more exons were genotyped in all 93 individuals from the 18 Jewish families. There were a total of 273 informative markers added to the haplotype map. Combining the MRR and extended MMR screen, 73 microsatellite, 47 SNPs from dbSNP, and 485 amplicons with one or more exons were sequenced and genotyped in the 93 individuals from the 18 PROGRESS Jewish families. However, there were still over 40 regions with gaps between informative markers over 0.3 Mb. Genotyping a high density of SNPs in most of the family members using the new SNP-typing platforms was obviously a useful approach.

The Affymetrix 100K SNP chips have a SNP every 50 kb on average. On chromosome 7 alone, there are 7,069 SNPs. For the Xba240 and Hind240 SNP chips, 86 and 77 individuals from the 18 Jewish families were genotyped (Months 13-18). The average call rate for Xba240 was 98.2% (91.1-99.5%) and 98.7% (90.5-99.7%) for the Hind240 chip. Not all SNPs were informative in the Jewish families. There were 678 SNPs with a minor allele frequency of 0%, and 802 SNPs with a minor allele frequency between 0-5%.

The Merlin linkage analysis program was used to build the chromosome 7 haplotypes within each family (Month 19). For the entire length of chromosome 7, there are 83.4 haplotypes on average. There were errors in the predicted haplotypes due to genotyping errors or other problems. These were identified by the presence of double-crossovers within a very short distance, and were corrected by hand. The affected haplotypes (cases) are the haplotypes shared by all the affecteds within a family, and the other haplotypes (controls) are all the remaining haplotypes in the families. Analysis scripts were designed and written to search the patterns in the affecteds file and test all patterns that occur more than once, determine the frequency of a pattern in the affecteds and others file, and calculate the p-value using Chi-squared or Fisher's exact (Months 20-21). Thus far, 22 regions where the frequency of a particular haplotype pattern occurred more frequently in the affected haplotypes than the other haplotypes have been investigated further (Months 21-24). For initial follow up, tagged SNPs were identified from the Caucasian HapMap data and genotyped in all 93 individuals from the 18 Jewish families. The Caucasian HapMap data was downloaded for each of the 22 regions. The density of SNPs is greater for the HapMap data and most of the Affymetrix Chip SNPs are in HapMap. SNPs were selected which differentiate HapMap haplotypes given the SNPs already genotyped in the families. Thus far, 113 HapMap SNPs have been genotyped in the 18 families. Only 2 SNPs were not informative in the Jewish families, which is a much higher success rate (98.2%) than during the previous phase of this project (62%). Two haplotype patterns were followed up in more detail after all the informative SNPs in the HapMap project were either on the SNP chip or were genotyped by sequencing. The coding regions for genes in these areas were sequenced until SNPs were discovered that indicated that these haplotype patterns were no longer significant and thus unlikely to be the founder HPC haplotype (Aim 5). As of January 2006, analysis

is still ongoing to identify the founder HPC haplotype by following up on the haplotype regions enriched in the shared affected haplotypes verses the other haplotypes in the families.

Aim 4. Prioritize candidate genes for mutation detection in the Ashkenazi Jewish HPC region by their location, expression pattern, and biological relevance (Month 12):

Task 4: Utilize web-based resources to prioritize candidate genes (Month 12). Candidate genes were prioritized before screening genes in the 5.7 cM MRR with 21 RefSeq genes, and in the two regions from the Affymetrix SNP Chip-based analysis. RefSeq was used via the UCSC genome browser (www.genome.ucsc.edu) to determine which genes were within the putative regions. As mentioned, 21 RefSeq genes were in the 5.7 cM MRR. There were seven RefSeq genes (77 exons) in the first additional region and only part of one gene with five exons in the second. UniGene was utilized to determine if there is evidence for expression in prostate tissue, and OMIM along with PubMed searches were used to determine what if known about the function of these genes/proteins.

Aim 5. Screen for disease-associated mutations and test findings in other families with linkage to the Ashkenazi Jewish HPC locus (Months 13-24): Although no definitive founder haplotype was identified. Over 580 exons were screened for mutations and SNPs for the analysis in Aim 3. Three regions targeted for complete exon sequencing are discussed here in Aim 5.

Task 5a: Design primers to amplify the coding regions and intron-exon boundaries for all candidate genes (Months 13). All exons and intron-exon boundaries were selected for sequencing in the 21 RefSeq genes in the 5.7 MRR (134 exons) and in the two regions from the Affymetrix SNP Chip-based analysis (82 exons). Many of the exons required multiple primer pairs before amplifying successfully. Fifteen exons within the 5.7 cM MRR would not amplify from genomic DNA even after testing multiple primer pairs. Some of these were in a small section of the MRR, which is duplicated within the genome.

Task 5b: Sequence the coding regions and intron-exon boundaries for all candidate genes (Months 14-21). Overall 201 out of 216 (93%) of the exons from these three selected regions were screened in all 93 individuals from the 18 Jewish families.

Task 5c: Determine if the variants identified segregate with the disease (Months 15-21). None of the variants discovered segregate perfectly with the disease.

Task 5d: Analyze intriguing variants in our other HPC families with linkage to the Ashkenazi Jewish HPC locus (Months 22-24). No putative disease-associated variant has been discovered yet.

Task 5e: Establish collaborations with other ICPCG members to test this variant in their Ashkenazi Jewish HPC families (Months 22-24). Although no disease-associated variant has been discovered, other members of the ICPCG are excited and interested in collaborating on this project when the putative mutation is identified, including William Isaacs from Johns Hopkins University, Kathleen Cooney from University of Michigan,

William Catalona from Northwestern University, as well as William Foulkes, Rosalind Eeles and Douglas Easton from the ACTANE Consortium.

KEY RESEARCH ACCOMPLISHMENTS

- The original 7q11-21 linkage result was supported by analysis of twelve additional markers spanning the 37.6 cM minimal critical region.
- The 7q11-21 linkage result was confirmed by analysis of a combined genome-wide scan of 36 Jewish HPC families (empirical *P*=0.006), including the original 17 PROGRESS families and 19 Jewish families from the JHU HPC study.
- The minimal recombination region for the 18 PROGRESS Jewish families was determined to be 5.7 cM with 21 RefSeq genes after genotyping twelve additional microsatellite markers.
- Almost all of the exons in the 21 RefSeq genes (119/134, 88%) in the 5.7 cM MRR were sequenced, and no obvious disease-associated mutation or founder haplotype was discovered.
- Key individuals from the 18 PROGRESS Jewish families were genotyped with the Affymetrix 100K SNP Chips (163 total chips).
- The Merlin linkage analysis program was used to build the chromosome 7 haplotypes within each family with the 7,069 SNPs from the SNP chips. An average of 83.4 haplotypes are present in all the families over chromosome 7.
- Analysis scripts were designed and written to search the patterns in the affecteds file and test all patterns that occur more than once, determine the frequency of a pattern in the shared affecteds and others file, and calculate the *P*-value using Chi-squared or Fisher's exact.
- Thus far, 22 regions where the frequency of a particular haplotype pattern occurred more frequently in the shared affected haplotypes than the other haplotypes have been investigated further by genotyping 113 potentially informative SNPs from the Caucasian HapMap project. Analysis is still ongoing to identify the founder HPC haplotype.
- All exons and intron-exon boundaries were selected for sequencing in three regions: the 5.7 MRR (134 exons) and two regions from the Affymetrix SNP Chip-based analysis (82 exons). Combined, 201 of the 216 exons (93%) from these three selected regions were screened in all 93 individuals from the 18 Jewish families.
- In total, 73 microsatellites, 47 SNPs from dbSNP, 113 HapMap SNPs, and 567 amplicons with one or more exons were sequenced in the 93 individuals from the 18 PROGRESS Jewish families. As well as, 28 microsatellites in the 18 JHU Jewish families.

REPORTABLE OUTCOMES

Primary Manuscripts and Presentations:

Friedrichsen DM, Stanford JL, Isaacs SD, Janer M, Chang B, Deutsch K, Gillanders E, Kolb S, Wiley KE, Badzioch MD, Zheng SL, Walsh PC, Jarvik GP, Hood L, Trent JM, Isaacs WB, Ostrander EA, Xu J (2004) Identification of a Prostate Cancer Susceptibility Locus on Chromosome 7q11-21 in Jewish Families. PNAS 101: 1939-1944.

"Refinement of the Prostate Cancer Susceptibility Locus on Chromosome 7 in Jewish Families" International Consortium for Prostate Cancer Genetics Spring 2005 Meeting, London, UK, 2005.

"Update on the Prostate Cancer Susceptibility Locus at 7q11-21 in Jewish Families" International Consortium for Prostate Cancer Genetics Spring 2004 Meeting, Ann Arbor, MI, 2004.

"Continuing analysis of a prostate cancer susceptibility locus on 7q11-21 in Jewish families." The 54th Annual Meeting of the American Society of Human Genetics, Toronto, Canada, 2004.

"In search of the founder haplotype for the prostate cancer susceptibility locus on 7q11-21 in Jewish families using the Affymetrix 100K SNP chips." The 55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, UT, 2005.

Other Manuscripts:

Stanford JL, McDonnell SK, **Friedrichsen DM,** Carlson EE, Kolb S, Deutsch K, Janer M, Hood L, Ostrander EA, Schaid DL (2005) Prostate cancer and genetic susceptibility: a genome scan incorporating disease aggressiveness. Prostate 66:317-325.

Friedrichsen DM, Hawley S, Shu J, Humphrey M, Sabacan L, Iwasaki L, Etzioni R, Ostrander EA, Stanford JL (2005) IGF-I and IGFBP-3 polymorphisms and risk of prostate cancer. Prostate 65:44-51.

Xu J, Dimitrov L, Chang BL, Adams TS, Turner AR, Meyers DA, Eeles RA, Easton DF, Foulkes WD, Simard J, Giles GG, Hopper JL, Mahle L, Moller P, Bishop T, Evans C, Edwards S, Meitz J, Bullock S, Hope Q, The Actane Consortium, Hsieh CL, Halpern J, Balise RN, Oakley-Girvan I, Whittemore AS, Ewing CM, Gielzak M, Isaacs SD, Walsh PC, Wiley KE, Isaacs WB, Thibodeau SN, Mc Donnell SK, Cunningham JM, Zarfas KE, Hebbring S, Schaid DJ, **Friedrichsen DM**, Deutsch K, Kolb S, Badzioch M, Jarvik GP, Janer M, Hood L, Ostrander EA, Stanford JL, Lange EM, Beebe-Dimmer JL, Mohai C, Cooney KA, Ikonen T, Baffoe-Bonnie A, Fredriksson H, Matikainen MP, Tammela TL, Bailey-Wilson J, Schleutker J, Maier C, Herkommer K, Hoegel J, Vogel W, Paiss T, Wiklund F, Emanuelsson M, Stenman E, Jonsson BA, Gronberg H, Camp NJ, Farnham J, Cannon Albright L, Seminara D. (2005) A Combined Genomewide Linkage Scan, of 1,233 Families, for Prostate Cancer–Susceptibility Genes Conducted by the International Consortium for Prostate Cancer Genetics. Am. J. Hum. Gen. 77:219-229.

Schaid DJ, Chang BL, **International Consortium For Prostate Cancer Genetics**. (2005) Description of the International Consortium For Prostate Cancer Genetics, and failure to replicate linkage of hereditary prostate cancer to 20q13. Prostate 63:276-290.

Narla G, Difeo A, Reeves HL, Schaid DJ, Hirshfeld J, Hod E, Katz A, Isaacs WB, Hebbring S, Komiya A, McDonnell SK, Wiley KE, Jacobsen SJ, Isaacs SD, Walsh PC,

Zheng SL, Chang BL, **Friedrichsen DM**, Stanford JL, Ostrander EA, Chinnaiyan AM, Rubin MA, Xu J, Thibodeau SN, Friedman SL, Martignetti JA (2005) A germline DNA polymorphism enhances alternative splicing of the KLF6 tumor suppressor gene and is associated with increased prostate cancer risk. Cancer Res 65: 1213-1222.

Friedrichsen DM, Malone KE, Doody DR, Daling JR, Ostrander EA. (2004) Frequency of CHEK2 mutations in a population based, case-control study of breast cancer in young women. Breast Cancer Res. 6:R629-35.

Employment and Research Opportunities:
Offered FTE Staff Scientist position at the National Institute of Health, National Human Genome Research Institute, Cancer Genetics Branch in Elaine Ostrander's group.

CONCLUSIONS

The final research accomplished has: 1) confirmed the 7q11-21 linkage result by analysis of an additional twelve microsatellite markers in the 37.6 cM region and a combined genome-wide scan of 36 Jewish HPC families (empirical *P*=0.006); 2) defined the minimal recombination region for the 18 PROGRESS Jewish families as a 5.7 cM interval with 21 RefSeq genes; 3) sequenced almost all of the exons in the MRR (119/134, 88%); 4) genotyped key individuals from the 18 Jewish families on the Affymetrix 100K SNP chips (163 total chips); 5) built the chromosome 7 haplotypes in each family with the 7,069 SNPs from the SNP chips; 6) designed and wrote analysis scripts to identify haplotype patterns enriched in the affecteds; 7) investigated 22 regions further by genotyping 113 potentially informative SNPs from the HapMap project; 8) generated a large volume of chromosome 7 genetic information with a total of 73 microsatellites, 7,069 chip SNPs, and 727 sequenced SNPs or amplicons with one or more exons.

REFERENCES

None.

APPENDICES

A: Original copy of journal article "Identification of a Prostate Cancer Susceptibility Locus on Chromosome 7q11-21 in Jewish Families." PNAS 101: 1939-1944.

B: Reprint of abstract "Continuing analysis of a prostate cancer susceptibility locus on 7q11-21 in Jewish families." The 54th Annual Meeting of the American Society of Human Genetics, Toronto, Canada, 2004.

C: Reprint of abstract "In search of the founder haplotype for the prostate cancer susceptibility locus on 7q11-21 in Jewish families using the Affymetrix 100K SNP chips." The 55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, UT, 2005.

D: Curriculum vitae

Identification of a prostate cancer susceptibility locus on chromosome 7q11–21 in Jewish families

Danielle M. Friedrichsen*, Janet L. Stanford†‡, Sarah D. Isaacs§, Marta Janer¶, Bao-li Chang‖, Kerry Deutsch¶, Elizabeth Gillanders, Suzanne Kolb†, Katherine E. Wiley§, Michael D. Badzioch††, S. Lilly Zheng‖, Patrick C. Walsh§, Gail P. Jarvik††, Leroy Hood¶, Jeffrey M. Trent***‡‡, William B. Isaacs§, Elaine A. Ostrander*§§, and Jianfeng Xu‖**

Divisions of *Clinical Research and Human Biology and †Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109; ‡Department of Epidemiology, School of Public Health and Community Medicine, and ††Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA 98195; §Department of Urology and Brady Urological Institute Research Laboratories, The Johns Hopkins Medical Institutions, Baltimore, MD 21287; ¶Institute for Systems Biology, Seattle, WA 98103; ‖Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC 27157; and **Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892

Contributed by Leroy Hood, December 15, 2003

Results from over a dozen prostate cancer susceptibility genome-wide scans, encompassing some 1,500 hereditary prostate cancer families, indicate that prostate cancer is an extremely heterogeneous disease with multiple loci contributing to overall susceptibility. In an attempt to reduce locus heterogeneity, we performed a genomewide linkage scan for prostate cancer susceptibility genes with 36 Jewish families, which represent a stratification of hereditary prostate cancer families with potentially increased locus homogeneity. The 36 Jewish families represent a combined dataset of 17 Jewish families from the Fred Hutchinson Cancer Research Center-based Prostate Cancer Genetic Research Study dataset and 19 Ashkenazi Jewish families collected at Johns Hopkins University. All available family members, including 94 affected men, were genotyped at markers distributed across the genome with an average interval of <10 centimorgans. Nonparametric multipoint linkage analyses were the primary approach, although parametric analyses were performed as well. Our strongest signal was a significant linkage peak at 7q11–21, with a nonparametric linkage (NPL) score of 3.01 ($P = 0.0013$). Simulations indicated that this corresponds to a genomewide empirical $P = 0.006$. All other regions had NPL P values ≥0.02. After genotyping additional markers within the 7q11–21 peak, the NPL score increased to 3.35 ($P = 0.0004$) at D7S634 with an allele-sharing logarithm of odds of 3.12 ($P = 0.00007$). These studies highlight the utility of analyzing defined sets of families with a common origin for reducing locus heterogeneity problems associated with studying complex traits.

In 2003, an estimated 220,900 men will be diagnosed with prostate cancer in the U.S., and 28,900 will die of the disease (1). Both epidemiological studies and segregation analyses confirm the existence of a genetic component to prostate cancer etiology. Two segregation analyses, both based on ascertainment of family history through probands treated with radical prostatectomy, show evidence for the dominant transmission of a rare high-risk allele (population prevalence of 0.3–0.6%), with carriers having an 88–89% risk of prostate cancer by 85 years of age, compared with 3–5% in noncarriers (2, 3). Carter *et al.* (2) suggest that the cumulative proportion of prostate cancer cases within the population attributable to high-risk susceptibility alleles is 43% for men ≤55 years of age, 34% for men ≤70 years of age, and 9% for men ≤85 years of age. By comparison, population-based studies from Sweden (4) and Australia (5) estimate a higher population prevalence of carriers (1.1–1.67%) and a lower lifetime incidence (63–79%). They suggest also that 23% of all prostate cancer cases diagnosed at <65 years of age may be due to inherited mutations in susceptibility genes (4).

These observations have motivated a large body of work aimed at finding susceptibility genes involved in hereditary prostate cancer (HPC). The scan of Smith *et al.* (6) in 1996 highlighted regions of chromosome 1q24–25, 4q27, and Xq27–28 as containing prostate cancer loci, with the result at 1q24–25 being statistically significant. A maximum multipoint logarithm of odds (LOD) score of 5.43 under the assumption of heterogeneity was observed, with 34% of families predicted to be linked. Several replication studies were published subsequently, with some confirming the initial findings (7–9), whereas others could not (10–14). A large metaanalysis of 772 families provided weak evidence overall, suggesting that about 6% of families could attribute their disease to *HPC1* (15). Several studies focus on *RNASEL* as a candidate gene for the *HPC1* locus (16), but attempts to demonstrate that mutations in *RNASEL* are solely responsible for the initial findings of linkage at 1q24–5 have been inconclusive (17–23).

Subsequent scans have identified loci on chromosomes 1p (*CAPB*) (24, 25), 1q (*PCAP*) (11), 20q (*HPC20*) (26), Xq (*HCPX*) (27), and *HPC2/ELAC2* on chromosome 17p (28) and the *MSR1* gene on chromosome 8p (29, 30). Other loci of interest have been reported on chromosomes 19p (31), 19q (32, 33), and 16q (34). As with *HPC1*, attempts at confirmation have proven inconclusive for *HPC20* (35, 36), *PCAP* (14, 37, 38), *HPCX* (39, 40), and *CAPB* (14, 41), as well as for the *HPC2/ELAC2* and *MSR1* genes (42–46).

In late 2003, eight additional genomewide scans for prostate cancer susceptibility loci were reported, including our own studies of 254 Prostate Cancer Genetic Research Study (PROGRESS) and 188 Johns Hopkins University (JHU) families (47, 48). The aggregate results are summarized in a review by Easton *et al.* (49). The eight scans include 1,292 families with multiple cases of prostate cancer. Across all studies, 11 peaks with LOD scores >2 were observed, identifying regions on chromosomes 2–7, 9, 16, 17, 19, and 20. No chromosomal region was reported as significant at the LOD ≥2.0 level by more than one study, and only one LOD score ≥3.0 was reported (49).

Given the extreme locus heterogeneity associated with HPC, we have sought ways to define homogeneous subsets for refined analyses. One approach is to evaluate families from relatively isolated populations with a limited number of founders. One well defined population, which meets these criteria and has proven useful for genetic studies of other cancer susceptibility genes, is of Americans of Ashkenazi Jewish descent (50). For instance, founder mutations within the *BRCA1*, *BRCA2*, and *MSH2* genes have been identified in studies of Ashkenazi Jewish individuals (51–55). Thus, we hypothesize that genomewide linkage analyses of HPC families of Jewish descent will increase locus homoge-

Abbreviations: HPC, hereditary prostate cancer; JHU, Johns Hopkins University; LOD, logarithm of odds; HLOD, heterogeneity LOD; Mb, megabases; NPL, nonparametric linkage; PROGRESS, Prostate Cancer Genetic Research Study.

‡‡Present address: Translational Genomics Research Institute, Phoenix, AZ 85004.

§§To whom correspondence should be addressed at: Division of Human Biology, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, P.O. Box 19024, Seattle, WA 98109-1024. E-mail: eostrand@fhcrc.org.

neity and our ability to find true susceptibility loci. Toward that end, we have analyzed a genomewide scan of 36 Jewish families by combining 17 families from the Fred Hutchinson Cancer Research Center-led PROGRESS and 19 families collected by JHU. These data highlight a region of chromosome 7q11–21 with significant results.

Subjects and Methods

Prostate Cancer Family Collection. Seventeen families were collected as part of the Fred Hutchinson Cancer Research Center-led national PROGRESS. They derive from a larger dataset of 255 families ascertained from throughout North America and several other countries by advertising a toll-free number by means of public media, health-related publications, and the internet, as well as communications with urologists, other health-care professionals, and prostate cancer support groups (10). To be eligible for the PROGRESS, families were required to meet at least one of the following criteria: (*i*) have three or more first-degree relatives with prostate cancer; (*ii*) have three generations with prostate cancer, through either paternal or maternal lineage; or (*iii*) have two first-degree relatives with prostate cancer diagnosed before age 65 or be African American. Families from the parent PROGRESS were eligible for this study if they self-identified as Jewish in response to a question on religious preference on a baseline questionnaire, which also collected information on each family member's country of origin. All 17 families are of Central or Eastern European descent and are likely to be Ashkenazi Jewish.

Medical records and death certificates were obtained to confirm the diagnosis of prostate cancer. Of the 48 medical records received on the putatively affected men who were genotyped, 100% confirmed the self-reported prostate cancer diagnosis. Death certificates confirmed an additional 6 of the 15 unsampled prostate cancer diagnoses in the 17 PROGRESS families. Only one sampled affected man's medical records were not available to confirm the diagnosis. Because of the high accuracy of prostate cancer self-reporting, the individual for whom records were not available was considered affected.

The 19 JHU Ashkenazi Jewish families are a subset of 188 HPC families that were collected and studied at the Brady Urology Institute at Johns Hopkins Hospital (48). A majority of cases were ascertained through referrals generated in response to a letter distributed to 8,000 urologists throughout the country. Families were also identified from family history records of patients seen at Johns Hopkins Hospital for treatment of prostate cancer. The remaining families were respondents to articles in a variety of lay publications describing JHU prostate cancer family studies. To qualify for the study, each family was required to have at least three first-degree relatives affected with prostate cancer. Medical records verified prostate cancer diagnosis for each affected male studied. Families were queried as to their religious preference to establish Jewish status. Individuals indicating Jewish were further asked to specify Ashkenazi or not, and all 19 families self-identified as Ashkenazi Jewish. Age at diagnosis of prostate cancer was confirmed either through medical records or from two other independent sources.

In the 36 PROGRESS and JHU Jewish families, all 45 genotyped affected men from JHU and 45 of 49 PROGRESS genotyped men self-identified as Jewish. The four genotyped affected men who did not self-report as Jewish are members of distinct families. None of the families were bilineal. In addition, no excess of breast or breast and ovarian cancer was observed when the Jewish datasets were compared, respectively, with the larger PROGRESS ($n = 254$) and JHU ($n = 188$) HPC datasets. It should be noted, however, that on average the Jewish families from both datasets were smaller and hence had fewer relatives at risk for breast or ovarian cancer than the non-Jewish families.

For both the PROGRESS and JHU families, study forms and protocols were approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center and the Johns Hopkins Medical Institutions, respectively.

Genotyping. For both studies, genomic DNA was extracted from peripheral blood lymphocytes by using standard techniques (56). For the PROGRESS families, a total of 441 microsatellite markers were genotyped. Details of the PCR amplification, marker characteristics, and genotyping are described elsewhere (25, 47). Briefly, the average marker heterozygosity was 70%, and the average spacing between markers was 8.1 centimorgans. Genotyping data were checked for errors before analysis by using PEDCHECK (57), RELPAIR (58), and PREST (59). The JHU families were genotyped by using 406 microsatellite markers with an average intermarker spacing of 10 centimorgans and average heterozygosity of 80%. PCR conditions and genotyping methods are described elsewhere (48). For both datasets, all genotyping included the same Centre d'Étude du Polymorphisme Humain (CEPH) individual (1347-02) for quality control purposes.

To combine the two datasets, only markers present in the University of California, Santa Cruz genome browser April 2003 assembly (http://genome.ucsc.edu) were used (PROGRESS 421 markers, JHU 398 markers). Map order and distance were based on the University of California, Santa Cruz map. The markers from the other genomewide scan were given no genotypes for all individuals (0, 0). There were 26 markers in common in both scans. To avoid the problem of allele binning, these markers were treated as separate markers and given map distances <0.1.

Statistical Analysis. A genomewide linkage scan was performed by using nonparametric multipoint linkage analyses as the primary method of analysis because of the uncertainty regarding likely mode of inheritance (60). Parametric multipoint analyses were also performed. The computer program MERLIN, Version 0.9.8, was used to perform affected relative-pair linkage analyses (61). The estimated marker identical-by-descent sharing of alleles for the various affected relative-pairs was compared with the values expected under the null hypothesis of no linkage. Model-free allele sharing was evaluated by using the nonparametric linkage $(NPL)_{all}$ statistic. Allele-sharing LOD scores were then calculated based on the NPL_{all} statistic, with equal weight assigned to all families (62). *P* values associated with LOD scores were calculated assuming the NPL_{all} statistic was normally distributed and were not adjusted for multiple tests.

Empirical *P* values were calculated for the NPL_{all} scores by performing simulations. The MERLIN program was used to generate and analyze 1,000 replicates of the entire genome from the original dataset of 36 Jewish families.

For the parametric analysis, a dominant two-liability class model was used. This model is based on the model used by Smith *et al.* (6) and assumes dominant inheritance of a disease allele with a frequency of 0.003. The two-liability class model is an affecteds-only analysis where the affection status of all unaffected men and women is assumed to be unknown. A maximum-likelihood approach was used to estimate the proportion of linked families by maximizing the admixed LOD score (heterogeneity LOD, HLOD), as implemented in the computer program GENEHUNTER, Version 2.0 (63, 64).

Results

The 36 Jewish HPC families have the following characteristics. There are 161 individuals genotyped, and, of the 149 affected men, 94 are genotyped. The mean age at diagnosis is 64.8 years, ranging from 38 to 81 years, and families contain from 2 to 10 genotyped individuals.

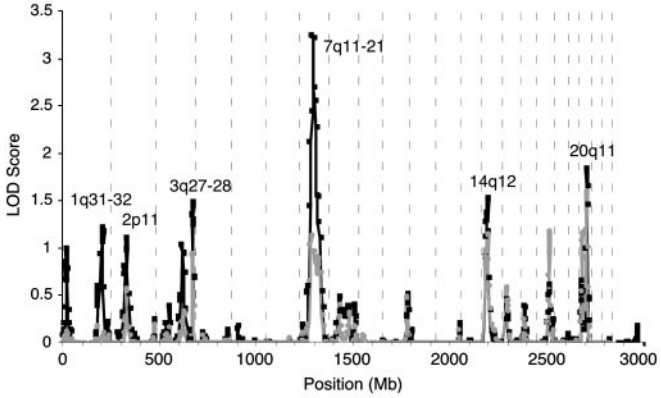

Fig. 1. Initial genomewide scan results for the 36 Jewish families. Allele-sharing LOD scores implemented by MERLIN are indicated by black squares, and HLOD scores from multipoint parametric analysis using the two-liability class model analyzed with GENEHUNTER are indicated by gray circles. Vertical dashed lines separate the chromosomes. Mb, megabases.

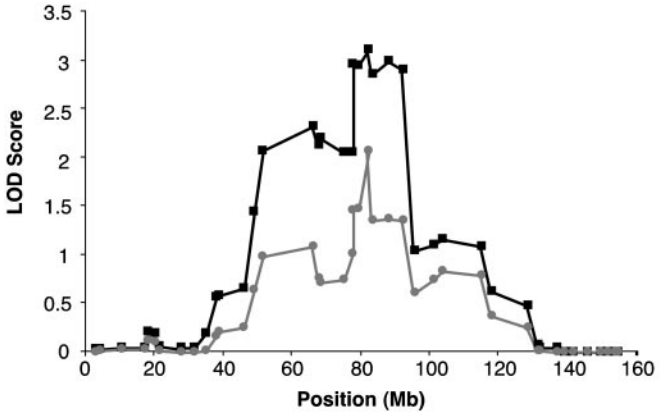

Fig. 2. Fine mapping multipoint linkage results on chromosome 7. Allele-sharing LOD scores implemented by MERLIN are indicated by black squares, and HLOD scores from multipoint parametric analysis using the 2-liability class model analyzed with GENEHUNTER are indicated by gray circles.

We performed a combined genomewide linkage analysis of the 36 families and identified a region of significant linkage on chromosome 7q11–21 (Fig. 1 and Table 1). The NPL *P* value was <0.01 for nine consecutive markers (D7S1818–D7S630) spanning 29.2 centimorgans (38.9 Mb). The maximum NPL score was 3.01 ($P = 0.0013$) at marker D7S502. Simulation studies find a genomewide probability of this NPL score of 0.006 in these data, thus, the genomewide *P* value is 0.006. The multipoint HLOD score was positive for the dominant two-liability model (HLOD = 1.14).

Our analysis of 36 Jewish families also highlighted regions on chromosomes 1q31–32, 2p11, 3q27–28, 14q12, and 20q11 with *P* values of 0.02 to 0.06. The strongest of these was at 14q12 ($P = 0.02$). Other minor peaks with an NPL *P* value ≤0.06 include 1q31–32 ($P = 0.06$), 2p11 ($P = 0.06$), 3q27–28 ($P = 0.03$), and 20q11 ($P = 0.04$) (Table 1). The dominant multipoint HLOD scores at 14q12 and 20q11 were 1.15 and 1.59, respectively, when the two-liability class model was used. For all of the other minor peaks, the multipoint HLODs were <1.0.

To refine the region of interest, we genotyped additional markers within the chromosome 7 peak and found support for the initial finding (Fig. 2 and Table 2). The final dataset included 11 markers across the 7q11–21 peak where genotypes are available from both JHU and PROGRESS datasets, including three markers not present in either original genomewide scan. With the inclusion of these markers, the NPL *P* values were <0.01 from D7S1818 to D7S630 as previously observed, and the maximum NPL score was 3.35 ($P = 0.0004$) at D7S634 with a corresponding allele-sharing LOD of 3.12 ($P = 0.00007$). Additionally, the multipoint HLOD generated by using the two-liability class model was 2.06. After stratifying by mean age at diagnosis, the NPL scores were 2.30 ($P = 0.011$) in 18 younger-age-at-diagnosis families (mean age <65) and 3.27 ($P = 0.0005$) in the 18 older-age-at-diagnosis families (mean age ≥65), indicating that although the older-age-at-diagnosis families account for most of the result, both younger- and older-age families contribute to the peak at 7q11–21. We had insufficient data from the older generations in many of the 36 families to accurately stratify by inheritance pattern (i.e., evidence of male-to-male transmission).

Finally, we considered the degree to which Jewish families accounted for results on chromosome 7q reported in our most recent genomewide scans (47, 48). In the case of the 254 PROGRESS families, we reported an HLOD of 2.25 (LOD = 1.55) at marker D7S2212 on 7q21 using a recessive parametric model. Furthermore, we noted an NPL result of 1.79 ($P = 0.038$) in this same region. Analysis of the 237 non-Jewish families from the PROGRESS dataset yielded an NPL score of 1.11 ($P = 0.134$), suggesting that the majority of the initial NPL result in the genomewide scan for the PROGRESS families can be accounted for by the Jewish families. In the genomewide study of 188 JHU families by Xu *et al.* (48), the strongest result on 7q was an allele-sharing LOD of 1.63 with marker D7S486. This result is at 7q22, which is ≈27 Mb from the region at 7q21 defined here. When 17 of the 19 JHU Ashkenazi Jewish families were analyzed by using D7S486, the allele-sharing LOD was only 0.04, suggesting that the JHU Ashkenazi Jewish families do

Table 1. Initial linkage results with NPL *P* values ≤0.06

	Nonparametric analysis				Parametric analysis*	
Chromosome	Peak marker	NPL	Position, Mb	*P*	HLOD	Position, Mb
1q31–32	D1S1660	1.53	195.1	0.06	0.10	211.8
	D1S413		195.1			
2p11	D2S2333	1.60	85.5	0.06	0.57	88.3
3q27–28	D3S1262	1.84	187.6	0.03	0.94	189.0
	D3S1580		189.9			
7q11–21	D7S502	3.01	66.5	0.0013	1.14	51.5
14q12	D14S1040	2.05	30.2	0.02	1.15	30.2
	D14S297		30.5			
20q11	D20S195	1.80	32.5	0.04	1.65	30.8

*Dominant parametric HLOD scores generated by using the two-liability class model.

Table 2. Chromosome 7 fine mapping linkage results

Marker	Position, Mb	Gap, Mb*	Nonparametric analysis		Parametric analysis†
			NPL	*P*	HLOD
D7S510	38.90	1.06	1.15	0.12	0.26
D7S519	45.82	3.28	2.03	0.02	0.65
D7S1818	49.10	2.36	2.48	0.007	0.99
D7S1830	51.46	15.00	2.62	0.004	1.09
D7S502‡	66.46	1.49	2.75	0.003	0.76
D7S3046‡	67.95	0.51	2.78	0.003	0.71
D7S2435‡	68.46	6.52	2.75	0.003	0.74
D7S2518‡	74.98	2.49	2.74	0.003	1.01
D7S669‡	77.47	0.26	3.07	0.0011	1.46
D7S2204‡	77.73	1.72	3.08	0.001	1.48
D7S634‡	79.45	2.95	3.35	0.0004	2.06
D7S2212‡	82.40	0.99	3.26	0.0006	1.36
D7S820‡	83.39	4.65	3.35	0.0004	1.36
D7S630‡	88.04	4.36	3.3	0.0005	1.36
D7S657‡	92.40	3.26	2.02	0.02	0.61
D7S821	95.66	5.59	1.93	0.03	0.75

*Distance from previous marker.

†Dominant parametric HLOD scores generated by using the two-liability class model.

‡Markers with genotypes available from both JHU and PROGRESS families.

not contribute significantly to the 7q22 result reported previously (48).

Discussion

We present here a genomewide scan for prostate cancer susceptibility loci in Jewish families, representing a well defined, isolated population. Only one significant linkage result was identified, on 7q11–21, although there were five other nonsignificant linkage peaks. The clear indication of only one susceptibility locus in these 36 Jewish families highlights the utility of this approach when investigating a disease with extreme locus heterogeneity, such as prostate cancer. Indeed, at this point in time, susceptibility loci have been identified on nearly every human chromosome (49).

The initial linkage result at 7q11–21 was a maximum NPL = 3.01 ($P = 0.0013$). Simulations indicated that this corresponds to a genomewide empirical $P = 0.006$. This initial finding was supported (NPL = 3.35, $P = 0.0004$) when additional markers in the region were genotyped. For both the initial and fine mapping linkage results, the multipoint HLOD scores (1.14 and 2.06, respectively) are less significant than the NPL results, presumably because of misspecification of the genetic models used in the parametric linkage analysis. For this heterogeneous disease, the parametric models were developed by using segregation analyses of populations that were not specifically Jewish (2–5). Therefore, these models are unlikely to accurately define prostate cancer genetics in the Jewish families.

Other studies have reported results of interest adjacent to 7q11–21, although none has been statistically significant. In an analysis of 326 affected sib pairs, Goddard *et al.* (65) modeled age as a covariate and observed a LOD score of 1.68 with markers overlapping the peak defined here at 7q11–21, with markers D7S3046 and D7S2204. In a previous study of the same affected sib-pair dataset, Witte *et al.* (32) identified a prostate cancer aggressiveness loci ≈44 Mb away at 7q31–32, by using Gleason score as an outcome variable. Their result at 7q31–32 ($P = 0.0007$) was one of the three strongest signals in the entire study. Fine-scale mapping by the same group (66) and a separate analysis of 100 German HPC families (67) support the finding at 7q31–32. We believe the locus defined by studies at 7q31–32 is distinct from the result reported here at 7q11–21. In 254 PROGRESS HPC families, we noted an NPL score of 1.77 ($P = 0.028$) with marker D7S1824 at 7q31–32 (47). However, the NPL score in the 237 PROGRESS families that did not self-identify as Jewish was 1.64 ($P = 0.05$), suggesting that the Jewish families do not contribute to the aggressiveness locus at 7q31–32. Furthermore, 7q31–32 is ≈44 Mb from the locus defined here at 7q11–21.

The strongest result reported previously at 7q11–21 was that described by our own analyses of 254 PROGRESS HPC families. Janer *et al.* (47) observed a peak LOD score at marker D7S2212 on 7q21 by using a recessive parametric model, with an HLOD of 2.25 (LOD = 1.55). Stratification of the dataset by age at diagnosis only slightly improved this result. In 214 families with a median age at diagnosis of 56–72 years, the HLOD was 2.41 at D7S2212 (LOD = 1.68). Analysis of the 31 HPC families with multiple breast and/or ovarian cancer identified an HLOD of 2.21 at D7S2204 (LOD = 2.15), where both the LOD and HLOD were 1.96 in the 15 HPC ovarian cancer families alone. In addition, analysis of the 237 families from the PROGRESS that did not self-identify as Jewish yielded an NPL score of 1.11 ($P = 0.134$) compared with 1.79 ($P = 0.038$) in the entire dataset, suggesting that the small number ($n = 17$) of PROGRESS Jewish families contribute disproportionately to the NPL result in the full dataset ($n = 254$). The co-occurrence of the multiple breast and/or ovarian cancer linkage result with the 7q11–21 result in the Jewish HPC families suggests that the 7q11–21 locus is important for a larger subset of HPC families than those who self-identify as Jewish.

The joint genomewide scan reported here also highlighted regions on chromosomes 1q31–32, 2p11, 3q27–28, 14q12, and 20q11 with *P* values of 0.02–0.06. Other studies have reported linkage peaks in some of these regions. On chromosome 20, we observed a minor peak at 20q11, which is >10 Mb from the *HPC20* locus defined by Berry and colleagues (26). At 2p11, Gibbs *et al.* (25), in an analysis of 94 PROGRESS families, observed a LOD score of 1.58 in families with a mean age at diagnosis of <65 years. In the study of all 254 PROGRESS families, an HLOD of 1.47 is reported at D14S1280 near the 14q12 peak observed in the 36 Jewish families described here (allele-sharing LOD = 1.38 at D14S1280) (47). Finally, our joint result at 1q31–32 is very near the *HPC1* locus, located at 1q24–25. *HPC1* was initially reported by Smith *et al.* (6) (HLOD = 5.43) when analyzing a set of 91 families, 79 of which were from JHU and 12 of which were from Sweden. Recently, Xu *et al.* (48) reported the analysis of 17 of the 19 JHU Ashkenazi Jewish families described here at *HPC1*, demonstrating that the highest allele-sharing LOD score was 1.70 at 1q31–32 (D1S413). By comparison, in the combined analysis of 36 Jewish families from PROGRESS and JHU, the maximum NPL score was 1.53 ($P = 0.06$) and the maximum allele-sharing LOD was 1.22.

RNASEL has been proposed as a candidate gene for the *HPC1* locus (16), and as with other candidate genes, replication studies have proven inconclusive (17–23). Although the *RNASEL* 471delAAAG mutation was found to be associated with prostate cancer in Ashkenazi Jews in one study (18), this was not confirmed in another similar study (21). Only 1 individual of 161 in the 36 Jewish families described here carries the 471delAAAG mutation (Avi Orr-Urtreger and Mariela Langlois, personal communication). Thus, the 471delAAAG mutation does not appear to be responsible for prostate cancer susceptibility in the 36 Jewish families described here. However, we cannot exclude the possibility that other *RNASEL* mutations could be responsible for the linkage result we observe at the *HPC1* locus.

Previous studies of the JHU Ashkenazi Jewish families have highlighted three additional loci of interest at 8p22, 10p15, and 20p13 (48, 68). None of these loci is supported in this joint analysis. The most provocative was the initial linkage report for

8p22, where a small number of Ashkenazi Jewish families (n = 11) contributed disproportionately to the linkage result with a maximum allele-sharing LOD of 1.31 compared with 1.39 for the remaining 133 non-Jewish Caucasian families (68). However, in this combined analysis of 36 Jewish families, no evidence for linkage was seen at 8p22 (allele-sharing LOD = 0.01). The differing results could reflect the small number of families available before the combined analysis.

Analysis of HPC families after stratifying by, or adjusting for, age at diagnosis has proven to be informative in previous linkage studies (reviewed in refs. 49 and 69–71). Stratification of the 36 Jewish families by mean age at diagnosis ($<$65 vs. $\geq$65 years) indicated that the older-age-at-diagnosis families contribute disproportionately to the chromosome 7 result (NPL = 3.27, P = 0.0005). However, the younger families also have a peak at 7q11–21 with an NPL = 2.3 (P = 0.011), suggesting that these families contribute to the result as well. Overall, these observations support the notion that the result reported here at 7q11–21 was not due to the contribution of only one or two families, and that replication of this result is likely in other datasets of Jewish HPC families. In addition, the differences in the younger- vs. older-age-at-diagnosis families is not surprising, as the median number of affected individuals with genotypes is 2.0 in the younger families and 3.0 in the older families. We note, however, that the median number of affected individuals reported per family is 4.0 for both the younger and older age at diagnosis families. Thus, the weaker linkage result obtained in the younger families probably reflects the reduced number of affected men who were genotyped in these families, and accordingly the reduced power of the analysis.

In summary, we performed a prostate cancer genomewide screen in Jewish families and identified a strong linkage result on chromosome 7q with a relatively small number of families. Our result suggests that reducing locus heterogeneity by grouping Jewish families of similar background is a useful strategy for linkage studies of complex diseases. Previously identified cancer susceptibility genes for which Ashkenazi Jewish founder mutations have been described include the *BRCA1*, *BRCA2*, and *MSH2* genes (51–55). Mutations in these genes are also strongly relevant for disease susceptibility in non-Jewish populations. Therefore, identification of the 7q11–21 gene is likely to be important in advancing our understanding of the etiology of this complex and common disease.

We especially thank the PROGRESS and JHU families for participating in this research. We thank Dr. Avi Orr-Urtreger and Mariela Langlois for providing the *RNASEL* sequencing information and Drs. Kyriacos Markianos and Joshua Akey for helpful comments on the PROGRESS linkage results. We also thank Toby Karyadi for PERL programming assistance. This research was supported by National Institutes of Health Grants RO1 CA80122 and RO1 CA089600 (to J.L.S.), RO1 CA78836 and K05CA90754 (to E.A.O.), and RO1 CA58236 (to W.B.I.); Fred Hutchinson Cancer Research Center Interdisciplinary Training Grant CA80416 (to D.M.F.); the Pew Biomedical Scholars Program (G.P.J.); and Department of Defense Grants DAMD17-98-1-8469 (to W.B.I.) and DAMD17-00-1-0087 (to J.X.).

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Continuing analysis of a prostate cancer susceptibility locus on 7q11-21 in Jewish families.
Danielle M. Friedrichsen[1], Janet L. Stanford[2], Marta Janer[3], Kerry Deutsch[3], Suzanne Kolb[2], Michael D. Badzioch[4], Gail P. Jarvik[4], Leroy Hood[3], Elaine A. Ostrander[1]. Division of Human Biology[1] and Public Health Sciences[2], Fred Hutchinson Cancer Research Center, Seattle, WA. Institute for Systems Biology[3], Seattle, WA. Department of Medicine, Division of Medical Genetics[4], University of Washington, Seattle, WA.

An estimated 220,900 men will be diagnosed with prostate cancer (PC), and 28,900 deaths will be attributed to the disease in the United States this year. Both epidemiological studies and segregation analyses confirm the existence of a genetic component to PC etiology, with an estimated 5-10% of all PC and 43% of early onset (≤55 years) disease being attributed to an inherited susceptibility. Recently, ten genome-wide scans have been published, analyzing 1,730 hereditary PC (HCP) families. The results of these studies confirm that a substantial amount of genetic heterogeneity exists in HPC.

One approach to reduce locus heterogeneity is to analyze Jewish families, which represent a more well-defined and genetically homogenous population. In our previously reported combined genome-wide scan of 36 Jewish HPC families from the Seattle-based PROGRESS study and Johns Hopkins University, we identified a region of significant linkage on chromosome 7q11-21, with an empirical *P* value of 0.006. Further resolution of the 7q11-21 locus in 18 Jewish PROGRESS families indicates that the minimal recombination region is 5.7 cM and contains approximately 20 RefSeq genes. This region expands to 29.2 cM and would contain over 100 RefSeq genes if three recombination events are used to define either boundary. Both SNPs and microsatellites are being evaluated in order to identify a potential founder haplotype. We have screened over 200 amplicons thus far and no obvious mutations have been found. This study highlights the utility of analyzing defined sets of families with a common origin for reducing locus heterogeneity problems associated with studying complex traits.

In search of the founder haplotype for the prostate cancer susceptibility locus on 7q11-21 in Jewish families using the Affymetrix 100K SNP chips.

Danielle M. Friedrichsen[1], Janet L. Stanford[2], Rick Wells[1], Marta Janer[3], Kerry Deutsch[3], Suzanne Kolb[2], Hau Hung[1], Michael D. Badzioch[4], Gail P. Jarvik[4], Pete Nelson[1], Leroy Hood[3], Elaine A. Ostrander[5]. Divisions of Clinical Research and Human Biology[1] and Public Health Sciences[2], Fred Hutchinson Cancer Research Center, Seattle, WA. Institute for Systems Biology[3], Seattle, WA. Department of Medicine, Division of Medical Genetics[4], University of Washington, Seattle, WA. Cancer Genetics Branch[5], NHGRI, National Institutes of Health, Bethesda, Maryland

Hereditary prostate cancer (HPC) is a genetically heterogeneous disease with evidence for multiple loci contributing to overall susceptibility. One approach to reduce locus heterogeneity is to analyze HPC in Jewish families, which represent a more well-defined and genetically homogenous population. We previously reported a combined genome-wide scan of 36 Jewish HPC families from the Seattle-based PROGRESS study and Johns Hopkins University, where we identified a region of significant linkage on chromosome 7q11-21, with an empirical *P* value of 0.006. Our strategy for isolating this prostate cancer susceptibility gene is to identify the founder haplotype surrounding the founder mutation in at least a subset of these Jewish families. Previously reported founder haplotypes in Jewish families for susceptibility genes in other diseases, like *BRCA1*/*BRCA2* or *MSH2*, have been 500 kb or larger. The Affymetrix 100K SNP chips have a SNP every 50 kb on average and chromosome 7 specifically has over 6,000 SNPs. The resolution of the 100K chips is potentially small enough to identify regions that may contain the founder haplotype. Currently, we are in the process of genotyping 90 members of the 18 Jewish PROGRESS families on the 100K SNP chips. This includes 51 affecteds and an additional 39 family members, which will be useful in determining chromosomal phase of the alleles. The data will be analyzed to determine regions where a specific haplotype pattern shared within families is also shared across several families for a large distance (greater than 500 kb) and where this pattern occurs more often in the shared affected haplotypes than in the other haplotypes in the families. After putative haplotypes are identified, SNPs from the HapMap project will be genotyped to confirm and further resolve the haplotypes identified. Finally, all exons in the remaining regions will be sequenced to discover the putative disease-associated mutation.

Danielle M Friedrichsen

Home: (206) 240-5768
Work: (206) 667-2748

809 NE 103rd St, Seattle WA 98125

dfriedri@fhcrc.org

TRAINING

Fred Hutchinson Cancer Research Center, Dept of Human Biology, Seattle, WA – 2001-Present
Postdoctoral Fellow

University of California, San Diego, Department of Biology, CA - 1995-2001
Ph.D. Molecular Genetics

Carnegie Mellon University, Department of Biology, Pittsburgh, PA - 1991-1995
B.S. with Honors in Biology

FELLOWSHIPS, GRANTS and AWARDS

DOD Postdoctoral Fellowship W81XWH-04-1-0083 – 2003-2005.
SPORE Pilot Project Grant "Development of Immortalized Cell Lines from Ashkenazi Jewish Hereditary Prostate Cancer Families" as Co-PI under Elaine Ostrander - 2004.
University of Washington/FHCRC Interdisciplinary Training Grant – 2002-2004
NIH Developmental Biology Training Grant - 1996-1999
Phi Beta Kappa - 1995
University, College and Departmental Honors - 1995

PRESENTATIONS (2002-Present)

"Refinement of the Prostate Cancer Susceptibility Locus on Chromosome 7 in Jewish Families" International Consortium for Prostate Cancer Genetics Spring 2005 Meeting, London, UK, 2005.

"Development of immortalized cell lines from Ashkenazi Jewish hereditary prostate cancer families" Pacific Northwest Prostate Cancer SPORE Advisory Board Meeting, Seattle, WA, 2005.

"Update on the Prostate Cancer Susceptibility Locus at 7q11-21 in Jewish Families" International Consortium for Prostate Cancer Genetics Spring 2004 Meeting, Ann Arbor, MI, 2004.

"Identification of a Prostate Cancer Susceptibility Locus on 7q11-21 in Jewish Families" Dual Mentor Research Symposium, Seattle, WA, 2003.

"Genetic Mapping of Prostate Cancer Susceptibility Genes" Dual Mentor Research Symposium, Seattle, WA, 2002.

POSTER PRESENTATIONS (1997-Present)

"In search of the founder haplotype for the prostate cancer susceptibility locus on 7q11-21 in Jewish families using the Affymetrix 100K SNP chips." The 55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, UT, 2005. * First author

"Identification and characterization of novel SNPs in CHEK2 in Ashkenazi Jewish men with prostate cancer." The 55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, UT, 2005.

"Prostate cancer and genetic susceptibility: a genome scan incorporating disease aggressiveness." The 55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, UT, 2005.

"Finding prostate cancer susceptibility genes using linkage based and candidate gene approaches." The 55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, UT, 2005.

"Genome wide linkage scan of aggressive prostate cancer: Results from the International Consortium for Prostate Cancer Genetics." The 55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, UT, 2005.

"Linkage analysis of familial prostate cancer with pedigree covariates by regression of nonparametric linkage scores." The 55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, UT, 2005.

"Linkage analysis of familial prostate cancer with recursive partitioning." The 55th Annual Meeting of the American Society of Human Genetics, Salt Lake City, UT, 2005.

"Continuing analysis of a prostate cancer susceptibility locus on 7q11-21 in Jewish families." The 54th Annual Meeting of the American Society of Human Genetics, Toronto, Canada, 2004. * First author

"Linkage analysis of chromosome 8p in the PROGRESS study families: Identification of a locus in older onset hereditary prostate cancer families." The 54th Annual Meeting of the American Society of Human Genetics, Toronto, Canada, 2004.

"Linkage heterogeneity in 254 hereditary prostate cancer (HPC) families." The 54th Annual Meeting of the American Society of Human Genetics, Toronto, Canada, 2004.

"Screen for prostate cancer susceptibility genes in 1,139 families: ICPCG combined genome-wide screen." The 54th Annual Meeting of the American Society of Human Genetics, Toronto, Canada, 2004.

"Failure to Replicate Linkage of Hereditary Prostate Cancer to 20q13 in the ICPCG Family Collection." The 54th Annual Meeting of the American Society of Human Genetics, Toronto, Canada, 2004.

"Linkage Analysis of Hereditary Prostate Cancer (HPC) to Xq27-28 in the ICPCG families." The 54th Annual Meeting of the American Society of Human Genetics, Toronto, Canada, 2004.

"Identification of a Prostate Cancer Susceptibility Locus in 37 Jewish Families." The 53rd Annual Meeting of the American Society of Human Genetics, Los Angeles, CA, 2003. * First author

"Associations of *IGF-I* and *IGFBP-3* Polymorphisms and Prostate Cancer Risk in a Case-Control Study of Middle Age Men." Oncogenomics 2002, Ireland, 2002. * First author

"The Induction of Two Putative bHLH Proteins by Brassinosteroids." 16th International Conference on Plant Growth Substances, Japan, 1998. * First author

"Brassinosteroids in *Arabidopsis* development." 8th International Conference on Arabidopsis Research, Madison, WI, 1997. * First author

RESEARCH EXPERIENCE

2004-2005: Co-mentors Elaine Ostrander and Peter Nelson, Human Biology, FHCRC

Hereditary prostate cancer is a genetically heterogeneous disease with evidence for multiple loci contributing to overall susceptibility. Our multi-center group of collaborators are investigating a variety of approaches aimed at reducing heterogeneity including analyzing prostate cancer in families from a specific founder population.

2001-2004: Elaine Ostrander, Human Biology, Fred Hutchinson Cancer Research Center
1996-2001: Joanne Chory, Plant Biology Laboratory, The Salk Institute
1991-1995: Elizabeth Jones, Department of Biology, Carnegie Mellon University
1994: Michael Lotze, Department of Surgical Oncology, University of Pittsburgh

TEACHING EXPERIENCE (2001-Present)

Genetics and Genetic Mapping Section of the ISTR Biotechnology training course. 2002-2003.

"DNA mutations and Cancer" Cancer Biology 101, Fred Hutchinson Cancer Research Center, 2002.

PUBLICATIONS

1 - Stanford JL, McDonnell SK, **Friedrichsen DM,** Carlson EE, Kolb S, Deutsch K, Janer M, Hood L, Ostrander EA, Schaid DL (2005) Prostate cancer and genetic susceptibility: a genome scan incorporating disease aggressiveness. Prostate 66:317-325.

2 - **Friedrichsen DM**, Hawley S, Shu J, Humphrey M, Sabacan L, Iwasaki L, Etzioni R, Ostrander EA, Stanford JL (2005) IGF-I and IGFBP-3 polymorphisms and risk of prostate cancer. Prostate 65:44-51.

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